FUNCTIONAL TESTS IN EXPERIMENTAL TARTRATE NEPHRITIS.[1]

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PATHOLOGY OF TARTRATE NEPHRITIS. Underhill, Wells, and Goldschmidt[2] succeeded in producing a severe form of acute nephritis in rabbits by subcutaneous injections of racemic tartaric acid. According to these observers, vacuolization of the epithelium of the convoluted tubules is the essential lesion in the earliest stages. Necrosis rapidly follows the vacuolization, so that nearly all the convoluted tubules may soon become necrotic. The glomeruli are practically uninjured. It was suggested by these investigators that tartrate nephritis offers a specially favorable opportunity for physiological studies, inasmuch as the convoluted tubules are practically all destroyed and the glomeruli left intact.

Pearce and Ringer,[3] from a study of tartrate nephritis in dogs, state that the most striking change is necrosis of the convoluted tubules, with fat in the loops of Henle and sometimes in the collecting tubules. They found exudative glomerular lesions in about one-half of the animals in which the tubular lesions were severe. Tartrate nephritis was purely tubular in only about one-half of their cases.

In our series of forty-five cases of experimental tartrate nephritis in rabbits the tubular lesions in the severe cases are essentially the same as those described by Underhill, Wells, and Goldschmidt. A few hours after injection of the tartrate the epithelium of the convoluted tubules may show extreme vacuolization. This condition was pronounced in one animal killed five and a half hours after the injection and in another killed two and a half hours after the injection. (See Figs. 1 and 2). The exact time of the first appearance of vacuolization was not determined. Necrosis and fatty metamorphosis begin the first day and advance rapidly. If the animal lives three or four days, fat may be a prominent feature. It is specially conspicuous in the convoluted tubules, but may be found in any tubule. Rarely there are some fat droplets in the glomeruli.

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[1] Aided by a grant from the Research Fund of the University of Minnesota.

[2] Tartrate nephritis, with special reference to some of the conditions under which it may be produced, Jour. Exper. Med., 1913, xviii, 322.

[3] A study of experimental nephritis caused by the salts of tartaric acid, Jour. Med. Research, 1913, xxix, 57.

When necrosis is well established there is usually no suggestion of the original vacuolated condition.

We have found a little hydropic degeneration (vacuolization) after the first day, but it seems to be a prominent lesion only during the first half day.

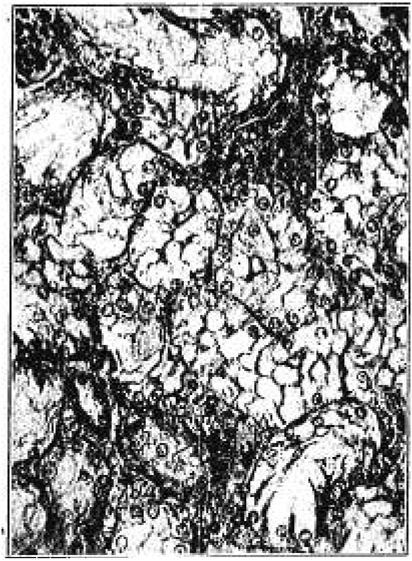

FIG. 1.—Kidney of rabbit five and a half hours after tartrate injection. Zenker, paraffin, hematoxylin, and eosin. Hydropic degeneration is very pronounced. The small tubules of the collecting type show little involvement. Photomicrograph.

The left kidney of a rabbit was removed seven and a half hours after the tartrate injection. This kidney showed extreme hydropic degeneration. Forty-eight hours after the nephrectomy the rabbit was killed. The right kidney showed necrosis of nearly all the convoluted tubules. No sign of hydropic degeneration was to be seen. It is therefore clear that the first lesion in tartrate nephritis is a hydropic degeneration, and that this condition gives place to fatty metamorphosis and necrosis in the later stages.

Hydropic degeneration involves all the convoluted tubules in all parts of the cortex. The first tubules to undergo necrosis are those near the capsule. The tubules in the deep part of the cortex next to the medulla become necrotic only in severe cases, though they may show marked degenerative changes. When all the convoluted tubules are necrotic there is usually an anuria so that no functional tests can be made. As a rule our functional tests were made on kidneys in which over half the convoluted tubules showed necrosis and the remainder fatty and granular degeneration.

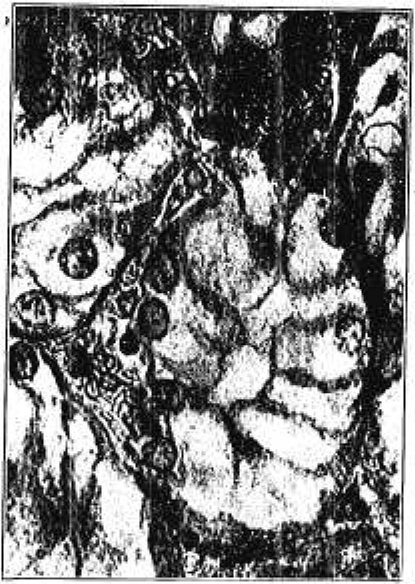

FIG. 2.—Small area from Fig. 1 under high magnification. Photomicrograph.

Some of the cases show swollen, opaque, cloudy kidneys. These have the gross features of cloudy swelling. When crushed specimens of fresh tissue are examined under high magnification it is seen that nearly all the tubules are granular. This granular condition is present in kidneys that are dark and reddish as well as in those that are pale and cloudy. The granules are least conspicuous when necrosis is most extensive. Of course, many of the granules are fat droplets. These may be identified by their insolubility in potassium hydroxide. But the albuminous granules are always present and frequently dominate the picture. These fade in potassium hydroxide but do not completely disappear. They are unlike the granules seen in the normal kidney. This granular condition of the epithelium is constantly present in tartrate nephritis of the mild as well as the severe types. It is best seen in crushed specimens of fresh tissue. It may be called a granular degeneration. The swollen, cloudy kidneys that show this microscopic appearance would usually be regarded as examples of cloudy swelling.

As regards the glomerular lesions, there were seven animals in our series of forty-five in which precipitated serum could be seen in some of the capsular spaces in every section. To what extent this indicates functional disturbance in the capsular epithelium we cannot determine. The glomerular tufts are often congested and rarely fat droplets can be demonstrated in them; but they are never found necrotic. And even in the most extreme cases of glomerular involvement there are always many in every section that are anatomically intact. It is probable that there is moderate glomerular injury in some cases of tartrate nephritis; but one may exclude these cases and use only those in which there are no anatomical glomerular lesions.

Schlayer and Takayasu[4] believe that the glomeruli may be functionally deranged even though they show no histological lesion. They therefore discard the histological evidence and estimate the glomerular function by testing the amount of contraction and dilatation of the renal vessels and the diuresis in response to stimulation. But as v. Monakow[5] has in part suggested, it is doubtful whether Schlayer's test is any better than the histological appearance. The capsular epithelium of the Malpighian corpuscle might be able to perform its functions even though the bloodvessels of the kidney do not respond to stimulation.

MATERIAL AND METHODS. Experimental tartrate nephritis has been studied in forty-five rabbits. In the latter half of our work we have used only racemic tartaric acid. In our first experiments Merck's dextrotartaric acid was used. Merck's preparation will produce a severe nephritis, but it is much less certain in its action than the racemic acid. It is also easier to produce a complete suppression of phenolsulphonephthalein excretion with the racemic acid. We have usually employed 0.4 gm. to 0.7 gm. of racemic tartaric acid neutralized with sodium carbonate and injected subcutaneously according to the method described by Underhill, Wells, and Goldschmidt. 0.3 gm. of the acid often produces complete suppression of the phthalein excretion, but much larger doses occasionally fail. In general a large dose is much more apt to produce a severe nephritis than a small dose. The animals were fed in the usual way during the experiments.

Some rabbits develop considerable resistance to tartrates. If they recover from the first dose much heavier doses are required subsequently to produce a nephritis. In one rabbit an initial dose of 0.6 gm. reduced the phthalein excretion to 6 per cent. for the first two hours. After the phthalein excretion had returned to normal, repeated doses of tartrate were given, some as high as 1.5 gm., but no signs of nephritis appeared.

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[4] Untersuchungen über die Funktion kranker Nieren, Deutsch. Arch. f. klin. Med. 1909, xcviii, 17.

[5] Beitrag zur Funktionsprüfung der Niere, Deutsch. Arch. f. klin. Med., 1911, cii, 248.

Only those animals have been used for further functional tests in which the nephritis produced was of such a degree as to cause complete or almost complete suppression of phthalein excretion. This condition is usually present as early as three hours or less after the injection of the tartrate, and continues until the death of the animal. An injection of phthalein was given every forty-eight hours during the experiment. Two rabbits that at one time showed complete suppression of phthalein, recovered completely.

PHENOLSULPHONEPHTHALEIN. Rowntree, Fitz, and Geraghty[6] have shown that this dye is excreted by the tubular epithelium in the frog's kidney. They have also shown that the elimination of phthalein is greatly reduced in experimental passive congestion whenever the congestion is made severe enough to produce marked tubular injury. In moderate passive congestion there is no marked change in phthalein excretion.

Rowntree and Geraghty[7] and Rowntree and Fitz[8] have given abundant clinical evidence that the excretion of phthalein is decreased in almost all forms of nephritis, and in general that the amount excreted corresponds to the degree of functional involvement of the kidney. Inasmuch as human nephritis never shows complete destruction either of all the tubules or of all the glomeruli it is difficult to decide from this material by what route the phthalein is eliminated.

Eisenbrey[9] found marked reduction of the phthalein excretion in experimental nephritis in dogs, produced by uranium nitrate and potassium chromate.

Austin and Eisenbrey[10] state that in experimental nephritis in dogs the anatomical picture bears no definite relation to the amount of phthalein excreted. These observers had no cases of zero phthalein elimination.

In our experiments twenty-three rabbits showed either no excretion of phthalein or a faint trace for the first two hours. All of these animals had very severe nephritis. In over half of these the animals were not allowed to die of nephritis but were killed immediately after a phthalein test, so that the corresponding anatomical picture could be studied. The lesions in the convoluted tubules are either severe hydropic degeneration, fatty metamorphosis, necrosis, or a mixture of fatty metamorphosis and necrosis. Granular degeneration is associated with all the changes just mentioned. The glomerular changes were discussed above. In tartrate nephritis a trace or a zero elimination of phthalein corresponds always to a very severe injury of the renal tissue. There may, however, be little or no necrosis.

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[6] The effects of experimental chronic passive congestion on renal function, Arch. Inter. Med., 1913, xi, 121.

[7] An experimental study of the functional activity of the Kidneys by means of Phenolsulphonephthalein, Jour. Pharmacol. and Exper. Therap., 1909–10, i, 579.

[8] Studies of renal function in renal, cardiorenal, and cardiac diseases, Arch. Inter. Med., 1913, xi, 258.

[9] A study of the elimination of phenolsulphonephthalein in various experimental lesions of the kidney, Jour. Exper. Med., 1911, xiv, 462.

[10] Experimental acute nephritis: The elimination of nitrogen and chloride compared with that of phenolsulphonephthalein, Jour. Exper. Med., 1911, xiv, 366.

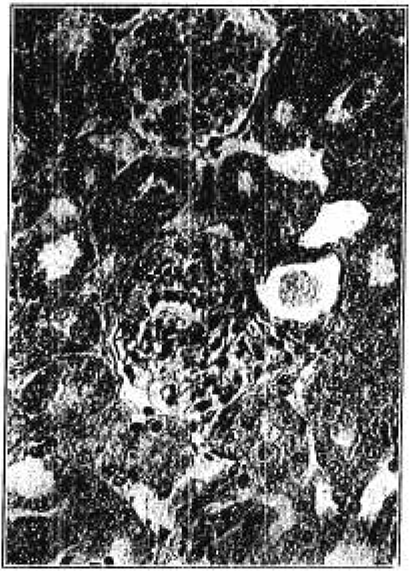

FIG. 3.—Kidney of a rabbit injected with tartrates the preceding day. Zenker, paraffin, hematoxylin, and eosin. The animal was killed immediately after a test which showed a 5.8 per cent. phthalein excretion for two hours. The epithelium is very granular, but there is no necrosis. Photomicrograph.

One of our animals was killed immediately after a test which showed 5.8 per cent. phthalein for the first two hours. All the epithelium was decidedly granular, but there was not much necrosis, vacuolization, or fat. (See Fig. 3.) Another animal, killed immediately after a test showing 6 per cent. elimination of the dye for two hours, had a rather marked hydropic degeneration. These two experiments indicate that different histological pictures may be associated with the same degree of functional derangement. Apparently the excessive accumulation of granules in the cytoplasm interferes with cell function even as vacuoles do.

Large quantities of urine, containing various normal constituents, may be excreted when the phthalein excretion is zero. In many of these cases the lesions are almost entirely tubular. It is, therefore, highly probable that phthalein is eliminated by the tubular epithelium.

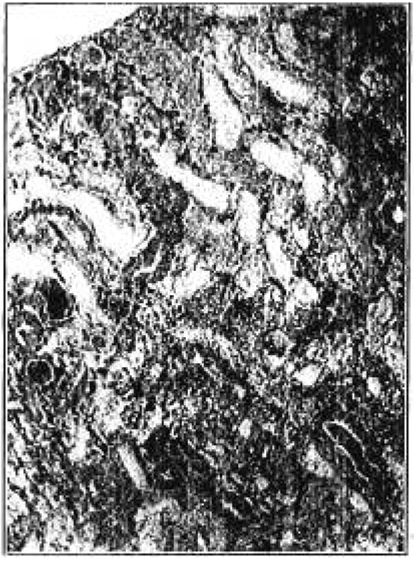

FIG. 4.—Kidney of a rabbit forty-eight hours after tartrate injection. Zenker, paraffin, hematoxylin, and eosin. The convoluted tubules are almost entirely necrotic. The intermediate tubules are granular,[9] but otherwise appear normal. This is the type of kidney we have used to a considerable extent in our functional tests. Photomicrograph.

A zero phthalein excretion does not necessarily mean a fatal prognosis in tartrate nephritis. Two rabbits showing this condition at one time have recovered completely so far as can be determined by phthalein excretion and general health. Rowntree[11] reports that he has seen 2 cases of chromate nephritis in rabbits showing zero phthalein excretion recover in a short time.

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[11] The study of renal function: The prognostic value of studies of renal function, AMER. JOUR. MED. SCI., 1914, cxlvii, 352.

Indigo-carmine. Rowntree, Fitz, and Geraghty[12] state that there is no additional information to be had from the use of indigo-carmine when phthalein is used, since it is excreted in amounts roughly corresponding to the amount of phthalein and is much more difficult to estimate colorimetrically. Rowntree and Geraghty[13] give a discussion of this test.

Our results also show parallelism between the excretion of phthalein and indigo-carmine. Two rabbits which showed a faint trace of phthalein for one hour were given indigo-carmine immediately. Only a trace of the dye was eliminated in each case. Three other rabbits which showed complete suppression of phthalein for the first hour likewise showed complete suppression of indigo-carmine injected immediately after the phthalein test. Phthalein and indigo-carmine are apparently excreted by the same structures in the kidney.

Methylene Blue. A discussion of this test is given by Rowntree and Geraghty[14] (p. 585). The excretion of this dye is said in general to run parallel to indigo-carmine. In some forms of chronic parenchymatous nephritis its excretion is said to be normal or exaggerated.

In our experiments one rabbit which showed a trace of phthalein for the first hour showed complete suppression of methylene blue. Two rabbits showing suppression of phthalein gave a trace of methylene blue. In two other rabbits both phthalein and methylene blue were completely suppressed. These results agree closely enough to prove that methylene blue is excreted in the same way as phthalein and indigo-carmine.

Lactose. De Bonis[15] injected a 1 per cent. solution of sodium fluoride into the ureter near the renal pelvis (Bottazzi's method). This procedure is said to destroy the medulla and all of the cortex except the glomeruli. More urine usually passes through the injured kidney than through the normal one. De Bonis[16] determined in this way that lactose may be excreted by the glomeruli. Rowntree, Fitz, and Geraghty[17] found that in the frog lactose may be excreted by the tubular epithelium. This experiment was performed by ligaturing the arterial supply of the glomeruli and then testing the renal function. (This method was first used by Nussbaum[18] in a study of renal function.) These authors also found that in experimental chronic passive congestion in dogs, lactose excretion is delayed one to two hours after intravenous injection. Marked delay in the excretion of lactose may occur when the phthalein output is normal.

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[12] Loc. cit. [13] Loc. cit. [14] Loc. cit.

[15] Giorn. intern. d. Science med., 1907, xxix, 446. (Cited from Rowntree and Fitz.)

[16] Experimentelle Untersuchungen über die Nierenfunktionen, Arch. f. Anat. u. Physiol., physiol. Abth., 1906, 271.

[17] Loc. cit. p. 126.

[18] Arch. f. d. ges Physiol., 1876, xvi, 179; xvii, 580.

Schlayer and Takayasu[19] used lactose as a vascular test in their experiments on rabbits. The normal kidney eliminates 1 gm. of lactose when injected intravenously in five and a half to seven and a half hours, average time six hours. In chromium, sublimate, and aloin nephritis lactose excretion is good in the earlier stages while the bloodvessels of the kidney react; but in the later stages when the bloodvessels no longer react its excretion time is prolonged to eight hours. No experiments showed suppression of lactose excretion.

Cases of human nephritis with delayed lactose excretion are regarded by Schlayer and Takayasu[20] as a vascular type. These authors believe that lactose and water are excreted by the glomeruli.

Rowntree and Fitz find lactose excretion delayed in all their human cases of nephritis or chronic passive congestion. In several cases they found a complete suppression of lactose excretion. These were all cases of severe nephritis. Lactose excretion in nephritis does not correspond to that of phthalein. The manner of its excretion is not yet determined.

To test the excretion of lactose we injected 3 gm. subcutaneously. The time required for the disappearance of sugar from the urine of normal rabbits after this injection varied from 13.5 to 23.5 hours in the five animals tested. The average time was twenty hours. Benedict's test for sugar was used. This is much longer than the time required to excrete 1 gm. when given intravenously. This period was found by Schlayer to average six hours in the normal rabbit.

Five animals in which the phthalein elimination was zero were injected subcutaneously with 3 gm. of lactose. The lactose disappeared from the urine in thirty-two, forty-two, forty-three, forty-eight, and forty-eight hours respectively—average 42.6 hours. This, it will be noted, is about twice the normal elimination time.

Several clinical cases reported by Rowntree and Fitz showed lactose excretion prolonged beyond twelve hours (2 gm. of lactose were injected intravenously in their cases). This period is also about twice the normal elimination time.

No case of lactose suppression has been encountered experimentally. Even when practically all the convoluted tubules are necrotic it is still excreted. Inasmuch as the destruction of the tubular part of the renal excretory apparatus is so complete in many cases of tartrate nephritis this condition affords specially clear proof that the glomeruli may excrete lactose. There is, however, no evidence in these experiments that the tubular epithelium cannot perform this function. The delayed excretion may mean that more must now be excreted by the glomeruli since the tubules are destroyed. There is no experimental evidence to explain the complete suppression of lactose observed in some of the clinical cases reported by Rowntree and Fitz. Some light might be thrown upon this question if a form of experimental nephritis could be produced in which lactose excretion was suppressed. Until further evidence is obtained we must agree with Rowntree that the manner of lactose excretion is not completely understood.

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[19] Untersuchungen über die Funktion kranker Nieren, Deutsch. Arch. f. klin. Med., 1909, xcviii, 17.

[20] Untersuchungen über die Funktion kranker Nieren beim Menschen, Deutsch. Arch. f. klin. Med., 1910-11, ci, 333.

POTASSIUM IODIDE. Frey[21] studied renal secretion by producing diuresis. He believed that the larger the amount of urine the nearer its composition would approach that of the glomerular excretion. He concluded from his experiments that the iodides are eliminated almost exclusively by the tubular epithelium.

Schlayer and Takayasu found that 0.025 gm. of potassium iodide injected intravenously into rabbits is completely eliminated in twenty-two to twenty-eight hours—average twenty-four hours. In experimental nephritis produced by chromate, sublimate, aloin, or uranium nitrate the iodide excretion is greatly delayed—forty to sixty hours. The excretion of potassium iodide may be greatly delayed, but it is never suppressed. The iodide is used by these authors as a test for the condition of the tubular epithelium, since they believe that it is excreted entirely by this part of the kidney tissue.

Von Monakow obtained indefinite results with potassium iodide. He does not regard it as a satisfactory test. In one of his clinical cases the autopsy revealed that nearly all the glomeruli were destroyed while the convoluted tubules showed only slight changes. In this case the iodide excretion was prolonged to one hundred and eighty hours. The prolongation of the excretion is much greater in this case than in those in which the lesions were mainly tubular.

Rowntree, Fitz, and Geraghty found that the excretion of potassium iodide is usually prolonged in chronic passive congestion of the kidney even in cases in which the phthalein output is normal.

Rowntree and Fitz found no constant relation between the degree of nephritis and the time of excretion of potassium iodide. They regard it, therefore, as of little value as a functional renal test.

Our tests were made by injecting 1 gm. of potassium iodide subcutaneously. Sandow's test was used for its detection in the urine. The time of elimination of this dose in four normal rabbits varied from 19.5 to 23.5 hours—average 21.5 hours.

In two rabbits in which phthalein excretion was permanently suppressed the elimination times for potassium iodide were 85.5

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[21] Das Glomerulusprodukt ist ein Blutfiltrat, Arch. f. d. gesam. Physiologie, 1912, cxxxix, 435.

hours and ninety-five respectively. Both animals died the night following the disappearance of the iodide from the urine. At autopsy one showed a considerable amount of necrosis of the tubular epithelium; the other showed extensive granular degeneration, fatty metamorphosis, and some necrosis.

Another rabbit in which the phthalein excretion was completely suppressed was killed sixty-five hours after the iodide injection. The iodide was still being excreted. At autopsy the majority of the convoluted tubules were found necrotic and the others were fatty and granular. The glomeruli were all anatomically intact.

These experiments show that iodide excretion is delayed in tubular nephritis. Schlayer and Takayasu obtained similar results, but interpreted them to mean that the iodide is excreted by the tubular epithelium and not by the glomeruli. Apparently they believe that the functional capacity of the epithelium is decreased but not completely suppressed in tubular nephritis.

But no one has yet reported an instance of suppression of iodide excretion except in anuria; whereas phthalein, which is almost certainly excreted by the tubules, is suppressed when the injury of the epithelium passes beyond a certain degree. Unless we believe that degenerated epithelium incapable of excreting phthalein, indigo-carmine, or methylene blue may still excrete iodide, we must admit that the glomeruli take part in the elimination of this substance. It seems more probable that the delayed excretion of iodide in our experiments is due to the fact that it must all be excreted by the glomeruli. Moderate injury of the glomeruli might further decrease the rate of excretion of iodide. The experimental results seem to us to afford good evidence that potassium iodide may be excreted by the glomeruli. The part that the tubular epithelium takes in the elimination of this substance is undetermined.

If we could produce experimentally a destruction of the glomeruli as complete as the destruction of the tubules in tartrate nephritis, valuable additional evidence could be obtained.

We have not made any functional tests with urea or sodium chloride. The discussion of these substances is therefore omitted.

HEMOGLOBIN. This test was performed as follows: 5 c.c. of washed red blood cells from a rabbit were hemolyzed with distilled water, and after the addition of 0.5 per cent. sodium chlorid injected into the ear vein of the rabbit to be tested. In normal rabbits the urine is almost black three or four hours after this injection. Two rabbits with tartrate nephritis, causing complete suppression of phthalein, each gave a slightly colored urine after this test. The benzidene test for blood was strongly positive in these urines, but the color of the urine was very much less dark than in the normal. It is therefore probable that hemoglobin is excreted mainly by the convoluted tubules, but that a small amount may pass through the glomeruli.

SUMMARY. By subcutaneous injections of tartaric acid it is possible to produce in rabbits a form of acute nephritis in which the great majority of the convoluted tubules are necrotic and the rest fatty and granular. The glomeruli and collecting tubules may be anatomically intact.

In kidneys of the type just described the excretion of phenolsulphonephthalein, indigo-carmine, and methylene blue is completely suppressed. The excretion time of lactose is over twice as long as normal, while the excretion time of potassium iodide may be four times the normal. Both potassium iodide and lactose are excreted by this type of kidney.

The evidence obtained suggests that phthalein, indigo-carmine, and methylene blue are excreted exclusively by the convoluted tubules and that potassium iodide and lactose may be excreted by the glomeruli. Hemoglobin is excreted mainly by the tubules, but may pass through the glomeruli slowly.

A REPORT OF THE TREATMENT OF CEREBROSPINAL SYPHILIS BY INTRASPINOUS INJECTIONS OF SALVARSANIZED SERUM.[1]

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SINCE the discovery of the Wassermann reaction and the luetin tests, scientific proof has been added to the clinical belief that such affections as tabes and general paresis are not parasyphilitic diseases but true luetic processes.

The discovery of the Spirocheta pallida in these diseases by Noguchi and others not only adds further proof, but establishes the fact that they are active syphilitic affections. One of the things that created the belief that these affections were parasyphilitic diseases was their lack of response, in most instances, to antisyphilitic treatment. The cause, we now know, is due to the peculiarity of the choroid plexus of the dura mater, being more or less impermeable to such drugs as mercury, potassium iodide, and salvarsan. We must admit, however, that occasionally clinical improvement follows the administration of salvarsan, mercury, and potassium iodide given in the usual manner, but

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[1] Read before the 116th Annual Meeting of the Medical and Chirurgical Faculty of Maryland, April 29, 1914.